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Gallbladder Color and Relative Fullness as a Field Technique for Estimating Time Since Last Feeding in Brook Trout

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Abstract

Brook trout (*Salvelinus fontinalis*) fingerlings were held for up to 13 days without food. Gallbladders sampled from these fish at successive times after their last meal showed filling and darkening in color directly related to time since last feeding. It is suggested that these changes may be useful in estimating the feeding state of fish in the field.

An estimate of the time since a fish has last fed is frequently of interest to fishery investigators and may be useful in certain pollution investigations (Statham et al. 1976). Usually it is sufficient to know whether or not the fish have been feeding on a regular daily basis and, if not, to know within a day or so the period of fasting; i.e., the timing of a spill resulting in a fish kill might be estimated based on the time since surviving fish went off feed due to the toxicant exposure. In this paper, we present a method for estimating the fasting time of sampled fish. The method is particularly advantageous to the field investigator because little time and equipment are required. The method involves only visual examination of the color of the gallbladder with its contents and its relative fullness. Although more precise measures of color darkening employing colorimetric instrumentation are available, the method described finds its utility in its expediency.

Materials and Methods

Well-fed, advanced fingerling brook trout (*Salvelinus fontinalis*) were held in a flowing-water hatchery trough at 8.3 C and starved for 13 days. Subsamples of these fish were taken for gallbladder examination at less than 1, 1, 2, 3, 5, 11, and 13 days (Fig. 1). All observations made less than 1 day after last feeding were pooled as half-day observations for curve-fitting calculations. The gallbladders containing bile were excised and placed in a well of a white porcelain reaction spot plate (in the field, white note paper would suffice). The color and degree of fullness of each bile-containing gallbladder was determined visually and recorded. The colors were assigned to five groups: colorless to yellow; yellow green; olive green; green; and dark green to dark blue-green. These color groups were assigned numerical bile color scores from 1 through 5 (Fig. 1) to yield semi-quantitative values for averaging within food-deprivation time categories.

Relationships between time without feeding and the associated color scores were determined by correlation analysis. Curve fitting was by the least-squares method, fitting an exponential equation of the form $y = aX^b$ where y equaled bile color score and X was the period (days) since last feeding (Brownlee 1965).

Results and Discussion

A direct relationship was found between the period of food deprivation and gallbladder color scores. A curve fitted to these data was described by the equation $y = 1.48X^{0.33}$ (Fig. 1). The fit of this line to the observed data had a coefficient of determination of $r^2 = 0.94$; i.e., 94% of the change in color score was associated with the length of time since last feeding.

Gallbladders examined within an hour or two after feeding were devoid or nearly devoid of bile. Feeding appears to initiate a gallbladder emptying in stomached fish (similar to that in mam-

mals) via a cholecystokinin reflex sequence after the passage of food through the gastrointestinal tract (Vigna and Gorbman 1977; Fange and Grove 1979). Bile was found in gallbladders only after a lapse of time of several hours after the last feeding. Little or no bile pigmentation was found in fish that had not eaten for a day, but with increased storage time the bile color darkened. A dark blue-green was the darkest color observed and was present only after 5 days or more of food deprivation. Mean color scores under 5 after a week or more without feeding included many individuals with a dark blue-green bile color. After 5 or more days without feeding, some fish had less deeply pigmented bile but the reason for this is not known. Possibly this "gallbladder emptying" by some individuals after long intervals without feeding may, under some circumstances, take place "spontaneously." Consequently, mean color scores, as presented in Fig. 1, may not be as diagnostic as pigmentation states within individual specimens. Figure 1 is presented primarily to indicate the close relationship between bile storage time and the color scores.

We suggest the following: (1) partially filled gallbladders with light-colored bile indicate recent feeding—within a day of examination; (2) filled gallbladders with yellow green through dark green bile indicate fasting from a few days to about a week; and (3) gallbladders with dark blue-green bile are indicative of several days of fasting—from as little as 5 days to more than a week.

The time-dependent darkening of stored bile probably was the result of progressive oxidation of bile pigments. As the bile enters the gallbladder initially, most of the porphyrins are in the yellow-gold bilirubin oxidation state, but after prolonged storage most eventually become the darker-colored, blue-green biliverdin (Bell et al. 1961; White et al. 1964; Schmidt and McDonagh 1972). Reabsorption of water and the subsequent concentration of the bile also may be a contributing factor to the darkening process (Mackay 1975).

The work reported here was done with brook trout and any application of this method to other fishes must be with reservation; i.e., it is known that some Cottidae possess blue-green serum color even before gallbladder storage of the bile begins (Low and Bada 1974). However, we believe this method is reasonably safe for other salmonids and probably for many other fishes as well.

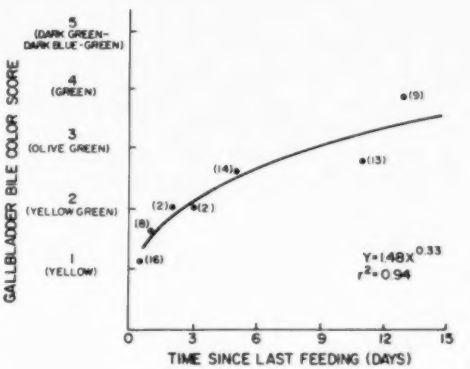

Figure 1. Relationship between mean gallbladder bile color scores (y) for brook trout and time since last feeding (X). (Numbers in parentheses are sample sizes.)

Smirnova (1977) reported similar bile color intensification with gallbladder storage time in common carp (*Cyprinus carpio*), and Love (1980) in codfishes (Gadidae).

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